PARIJS

ORSAY
Série A
N° d'Ordre : 1186

SUJET DE LA THESE

Etude du contrôle de la synthèse d'ADN chez un mutant de Bacillus subtilis thermosensible pour l'initiation de la réplication.

ARTICLE PRINCIPAL

recouvrant en partie la thèse de Doctorat ès-sciences naturelles enregistrée au C.N.R.S. sous le Numéro A.O 8786

Simone LAURENT née SEROR

soutenue le 20 Novembre 1973 à l'UNIVERSITE DE PARIS-SUD
Centre d'ORSAY

devant le Jury :

MM.	P. SCHAEFFER	Président
	F. GROS	Examinateur
	F. JACOB	"
	J. LEGAULT-DEMARE	"

RESUME DE LA THESE

Les bactéries sont capables de régler leur taux de synthèse d'ADN de telle sorte que la réplication du chromosome et la division cellulaire s'effectuent au même rythme. Le taux de synthèse d'ADN paraît être contrôlé par la fréquence avec laquelle de nouvelles fourches de réplication sont initiées, plutôt que par la vitesse d'avancement de ces fourches sur le chromosome. L'initiation semble donc jouer un rôle fondamental dans la régulation de la synthèse d'ADN

Le travail exposé ici se propose d'étudier certains aspects du mécanisme et du contrôle du processus d'initiation chez un mutant de B.subtilis altéré à haute température pour l'initiation de la réplication.

I.-Caractérisation du mutant Ts 37

Nous avons isolé un mutant de B.subtilis Ts 37, chez lequel la synthèse d'ADN est spécifiquement inhibée à haute température (1). Chez ce mutant, la synthèse d'ADN s'arrête 20 à 30 min. après le transfert de 30°C à 45°C, permettant ainsi une augmentation de l'ADN de 40 à 50 % , cependant, les synthèses d'ARN et de protéines continuent à un taux normal pendant environ 60 min. Un blocage de la synthèse protéique permet une synthèse résiduelle d'ADN équivalente.

Une expérience de transfert simultané de température et de densité montre que l'ADN répliqué à 45°C est largement enrichi en marqueur Méthionine (situé à la fin du chromosome), alors que l'ADN non répliqué à 45°C est très riche en marqueur Adénine (situé au début du chromosome). De plus, l'ADN transformant extrait d'une culture ayant séjourné à 45°C présente, pour les marqueurs situés à l'origine et à la fin du chromosome, une efficacité de transformation relative caractéristique de chromosomes complets. Ces résultats démontrent donc clairement que la synthèse résiduelle d'ADN observée à 45°C correspond à la terminaison des cycles de réplication en cours, alors que de nouveaux cycles ne sont pas initiés à cette température.

II - Réversibilité de l'inhibition de la synthèse d'ADN

Après un arrêt à 45°C, la synthèse d'ADN reprend si la température est abaissée à 30°C. La réplication ainsi que la division cellulaire sont alors synchronisées. Afin de savoir si la reprise de la synthèse d'ADN à 30°C exige une synthèse protéique, du chloramphénicol (150 µg/ml) est ajouté avant l'abaissement de la température. Dans ces conditions, un doublement de la quantité initiale d'ADN est observé (2). Au contraire, aucune synthèse d'ADN n'est possible, si le chloramphénicol est ajouté dès le début du séjour à 45°C. Des expériences de transfert de densité et d'analyse de la fréquence des gènes situés à l'origine et à la fin du chromosome montrent que la synthèse d'ADN observée après un abaissement de température en présence de chloramphénicol correspond à un cycle complet de réplication.

Une protéine thermosensible exigée pour l'initiation est donc synthétisée à 45°C sous une forme inactive. Cette protéine est réactivée après un abaissement de température et permet alors l'initiation d'un nouveau cycle de réplication.

III - L'initiation de la réplication exige une synthèse d'ARN

Afin de savoir si chez B.subtilis, de même que chez E.coli une synthèse d'ARN est nécessaire pour initier un nouveau cycle, nous avons utilisé le système précédemment décrit qui permet d'étudier l'initiation de la réplication du chromosome, en l'absence de synthèse protéique. Si, après un séjour à 45°C, la température est abaissée à 30°C en présence d'inhibiteurs de la synthèse d'ARN (rifampicine ou streptolydigine), la synthèse de l'ADN ne reprend pas (2). Ceci n'est pas dû à une inhibition directe de la synthèse d'ADN par ces inhibiteurs, puisque ces derniers, de même que le chloramphénicol, permettent une augmentation d'ADN d'environ 40 % quand ils sont ajoutés à une culture en croissance exponentielle.

IV - Régulation de la synthèse des protéines d'initiation

Après un ésjour à 45°, le mutant Ts 37 est capable d'initier un cycle de réplication si le retour à 30° est effectué en présence de chloramphénicol. Cependant, l'initiation du deuxième cycle de réplication exige une synthèse protéique, même après un séjour prolongé à 45°.

Les expériences effectuées afin de déterminer à quel moment apparaissent les protéines nécessaires pour initier le deuxième cycle montrent quelles peuvent être présentes dans les bactéries bien avant l'initiation de ce cycle (3). De plus, l'apparition de ces protéines est influencée par la longueur du séjour antérieur à 45° : quoique des cinétiques semblables de réinitiation soient observées à 30° après 30 minutes ou 65 min à 45°, la capacité à initier le deuxième cycle de réplication sans synthèse additionnelle de protéines apparait beaucoup plus tôt si le séjour à 45° est long.

Afin d'expliquer d'une part la limite de la capacité d'initiation acquise après un arrêt de la synthèse d'ADN, d'autre part l'influence de la durée de cet arrêt sur l'acquisition des protéines d'initiation lorsque les conditions permissives sont rétablies, nous sugggérons l'existence de deux protéines soumises à des mécanismes de régulation différents, toutes deux nécessaires pour l'initiation de la réplication. L'une d'elles synthétisée à un taux proportionnel au taux de synthèse protéique, peut être accumulée à 45° pendant l'inhibition de la synthèse d'ADN. La seconde protéine, au contraire, est synthétisée pendant une fraction limitée du cycle cellulaire -au début du cycle de réplication- et ne peut pas être accumulée dans les bactéries. L'association de ces deux protéines serait nécessaire pour initier un nouveau cycle de réplication.

La protection du matérill d'initiation contre l'effet dénaturant de la température, lorsqu'il est d'abord exposé à 30°, est compatible avec l'hypothèse proposée si on admet que la structure de la protéine thermosensible est stabilisée par son association avec un autre élément du complexe d'initiation.

Enfin, nous avons montré que la présence de protéines spécifiques et celle d'un ARN particulier sont insuffisantes pour induire l'initiation d'un nouveau cycle de réplication. Ceci suggère l'existence d'une étape supplémentaire : on peut envisager notamment que la terminaison d'un cycle provoque une modification de la structure du chromosome au niveau de l'origine, telle que le complexe d'initiation devienne fonctionnel.

1 - LAURENT, S.J., and F.S. VANNIER. 1973. Temperature-Sensitive. Initiation of Chromosome Replication in a Mutant of Bacillus subtilis. J. Bacteriol. 114 : 474-484.

2 - LAURENT, S.J. 1973. Initiation of Deoxyribonucleic Acid. Replication in a Temperature-Sensitive Mutant of B.subtilis. J. Bacteriol. 116 : 141-145.

3 - LAURENT, S.J. 1974. Control of Deoxyribonucleic Acid. Synthesis in a Bacillus subtilis Mutant Temperature sensitive for Initiation of Chromosome replication. J. Bacteriol. 117 : 329-336.

JOURNAL OF BACTERIOLOGY, Oct. 1973, p. 141-145
Copyright © 1973 American Society for Microbiology
Vol. 116, No. 1
Printed in U.S.A.

Initiation of Deoxyribonucleic Acid Replication in a Temperature-Sensitive Mutant of *B. subtilis*: Evidence for a Transcriptional Step

SIMONE J. LAURENT

Fondation Curie-Institut du Radium, Université Paris-XI, Bâtiment 110, 91405-Orsay, France

Received for publication 14 May 1973

A mutant of *Bacillus subtilis* unable to initiate a new round of replication at 45 C has been described. Here we show that inhibition of DNA synthesis in this mutant is reversible and that DNA synthesis is resumed at low temperature, even in the presence of chloramphenicol. Initiation of a new replication cycle thus can occur in the absence of protein synthesis. A thermolabile component required for initiation therefore appears to be synthesized at 45 C in an inactive form and can be activated at 30 C in the presence of an inhibitor of protein synthesis. Although resistant to chloramphenicol, the reinitiation of replication occurring after lowering the temperature is sensitive to rifampin and streptolydigin.

During bacterial growth, the rate of deoxyribonucleic acid (DNA) synthesis appears to be controlled by the frequency with which new replication forks are initiated, rather than by the migration speed of these forks along the chromosome (6, 17, 19, 26). Initiation of chromosome replication is blocked after an inhibition of protein synthesis (13, 14, 16, 17), whereas it can occur after an arrest of DNA synthesis (21).

We have previously described a mutant of *Bacillus subtilis* that is unable to initiate a new replication round at 45 C (15). This paper shows that DNA synthesis is resumed at 30 C, even in the presence of chloramphenicol, after a previous inhibition of DNA synthesis at 45 C. In this system, initiation of DNA replication can thus be studied in the absence of protein synthesis. We took advantage of this property to show that initiation of DNA replication in *B. subtilis* requires a transcriptional step, as appears to be the case also in *Escherichia coli* (12).

This paper will be part of a doctoral thesis to be submitted in 1973 to the Faculty of Sciences of the University of Paris-XI (Orsay).

MATERIALS AND METHODS

Bacterial strains. The mutant Ts37 was derived from strain 168 Thy^-, Trp^-, Ura^- (15). Strain 168 Mu 8u 5u 16 (Leu^-, Met^-, Ade^-) was used as a recipient in the transformation experiments.

Growth conditions. Bacteria were cultivated at 30 or at 45 C with aeration on a semisynthetic medium. Minimal Spizizen medium (24) was supplemented with glucose, 0.5%; casein hydrolysate, 0.02%; thymine, 5 μg/ml, tryptophan, 40 μg/ml, and uracil, 20 μg/ml. Growth was determined by variation in optical density at 570 nm.

Estimation of the amount of DNA synthesized. DNA was uniformly labeled by cultivation at 30 C for at least 10 generations in the presence of ^{14}C-thymine (C.E.A., France; specific activity 46.7 mCi/mmol) added at a concentration of 0.08 μCi per 5 μg per ml or in the presence of ^{3}H-thymine (C.E.A., France, specific activity 34 Ci/mmol) added at a concentration of 10 μCi per 5 μg per ml. Trichloroacetic acid-precipitable radioactivity was measured as previously described (15).

Labeling of DNA for density centrifugation. Bacteria were grown at 30 C in supplemented Spizizen medium in which $^{15}NH_4Cl$ (99.1% ^{15}N; A.P.C. Toulouse, France) and ^{2}H-glucose (*d*-glucose, Cd7; Merck Sharp & Dohme) were substituted for NH_4Cl and glucose. At the desired time, bacteria were diluted in 10 volumes of normal medium.

CsCl gradient. Cells were lysed by lysozyme, as described earlier (15). CsCl (8.05 g) was dissolved in 6.25 g of lysate. Centrifugation was carried out at 35,000 rpm for 60 h at 20 C in a Spinco 50Ti fixed-angle rotor. Seventy-eight to eighty fractions were collected. A sample of each fraction was deposited on a Whatman 3MM paper disk; the disks were treated according to Bollum (3), and the radioactivity was determined in a liquid scintillation counter.

Transformation experiments. Competent cul-

tures of strain 168 Mu 8u 5u 16 were obtained as previously described (15). DNA was prepared either by the method of Marmur (18) or by centrifugation of a crude bacterial lysate on a cesium chloride gradient. In the latter case, prior to use for transformation, the gradient fractions were dialyzed at 4 C for 36 h against two changes of 1 liter of 0.9% NaCl. Ade-Met ratios were normalized against the relative transformation frequencies of Ade and Met markers observed with the DNA extracted from an amino acid-starved culture of mutant Ts37.

RESULTS

Properties of the initiation mutant Ts37. The mutant Ts37 (15) has properties similar to some of the initiation mutants described in *E. coli* (1, 2, 9, 11, 23, 30). When a culture exponentially growing at 30 C is shifted to 45 C, DNA synthesis continues for about 30 min and stops, permitting a DNA increase of 40 to 50%. Proteins, however, are synthesized at a normal rate for 60 to 90 min. If the culture is returned to 30 C, inhibition of DNA synthesis is immediately reversed, and replication is resumed synchronously (15).

Temperature-sensitive protein is reversibly inactivated at 45 C. To determine whether protein synthesis is required for the resumption of DNA synthesis, chloramphenicol (150 μg/ml) was added to a mutant culture at the end of the 45 C period, 5 min before returning to 30 C. After a period of 35 min at 45 C, DNA synthesis is resumed when temperature is lowered in the presence of chloramphenicol (Fig. 1), thus permitting a doubling of the initial amount of DNA. The amount of DNA synthesized at 30 C is lower when the 45 C period is shortened (20 min), but remains constant and does not exceed a doubling if the 45 C period is extended from 35 min to 2 h.

These experiments suggest that the protein which is temperature sensitive in mutant Ts37 is synthesized at 45 C in an inactive form. When the cells are returned to 30 C, this protein is reactivated and permits the initiation of a new replication cycle, even in the presence of chloramphenicol. If this interpretation is correct, one should not observe any DNA synthesis upon return to 30 C, when protein synthesis is blocked during the exposure to 45 C. To test this prediction, chloramphenicol was added at the beginning of the 45 C period, and DNA synthesis was followed as before. As expected, the presence of chloramphenicol does not affect the residual DNA synthesis at 45 C, but completely inhibits DNA synthesis when bacteria are returned to 30 C (Fig. 1).

The following experiment shows that DNA synthesis occurring at 30 C in the presence of chloramphenicol represents one normal cycle. An exponential-phase culture of mutant Ts37 growing in heavy medium in the presence of ^{3}H-thymine was transferred to 45 C for 60 min; it was then returned to 30 C, and at the same time shifted to light medium containing ^{3}H-thymine and chloramphenicol. The DNA synthesized under these conditions was analyzed by a cesium chloride density gradient centrifugation. Practically all of the DNA present in the cells after a period at 30 C is of hybrid density (Fig. 2), as would be expected if all of the chromosomes had been completely replicated once in the presence of chloramphenicol. This was confirmed by measuring the relative frequency of origin and terminus markers by genetic transformation. In contrast with an exponentially growing culture, in which the Ade/Met ratio was approximately 2, this ratio became approximately 1 after a period at 45 C, and the same value was found for hybrid DNA formed when the temperature was lowered to 30 C in the presence of chloramphenicol, thus reflecting completed chromosomes.

Initiation of DNA replication is sensitive to drugs that inhibit transcription. There is

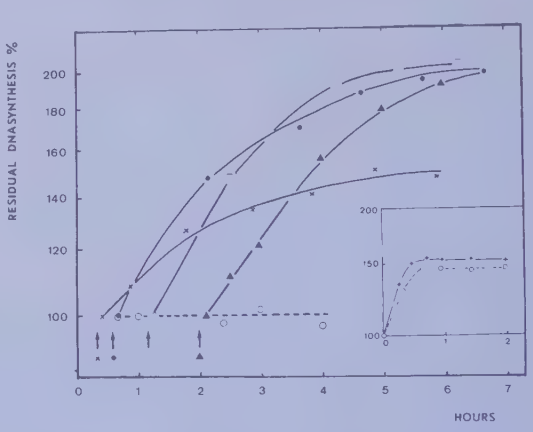

FIG. 1. *Reversibility of inhibition of initiation in the mutant Ts37. An exponential-phase culture of strain Ts37 incubated at 30 C in the presence of ^{14}C-thymine (0.08 μCi per 5 μg per ml) for 10 generations was divided into several parts. Four of them were shifted to 45 C, and chloramphenicol (150 μg/ml) was added after 20, 35, 70, and 120 min* (×, ●, □, ▲, *respectively); 5 min after the addition of chloramphenicol, the samples were returned to 30 C. The arrows indicate the time of addition of chloramphenicol. In a control sample* (○), *chloramphenicol was added immediately before the shift to 45 C and, after 40 min at high temperature, bacteria were returned to 30 C. Incorporation of ^{14}C-thymine into trichloroacetic acid-precipitable material was followed as described in Materials and Methods. Values are normalized to the values at the time of the shift back to 30 C, taken as 100. The insert shows DNA synthesis at 45 C in the absence* (+), *and in the presence of chloramphenicol* (○).

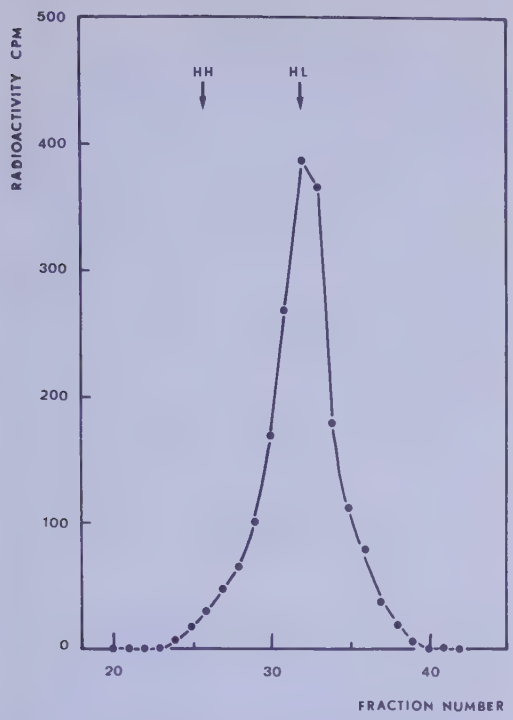

FIG. 2. *Cesium chloride density gradient centrifugation analysis of the DNA synthesized at 30 C in the presence of chloramphenicol after a previous period at 45 C. An exponential-phase culture of strain Ts37 grown in the presence of ^{3}H-thymine (10 μCi per 5 μg per ml) on supplemented Spizizen $^{15}N^{2}H$ medium was shifted to 45 C. After 1 h, chloramphenicol (150 μg/ml) was added; 5 min later, bacteria were diluted in 10 volumes of normal medium containing ^{3}H-thymine and chloramphenicol and were further incubated at 30 C. After 4 h, DNA was extracted and analyzed on a CsCl gradient as described in Materials and Methods. Positions of heavy (HH) and hybrid (HL) DNA labeled with ^{14}C-thymine and cocentrifuged with the assay are indicated on the graph.*

some evidence that the initiation process in *E. coli* involves a transcriptional step (12). To determine whether this is true also for *B. subtilis*, we used the system described above, which permitted us to study chromosome initiation in the absence of protein synthesis. A culture of the mutant Ts37 was transferred to 45 C for 60 min, and was returned to 30 C in the presence of rifampin or streptolydigin, drugs which inhibit ribonucleic acid (RNA) synthesis (5, 27). DNA synthesis is not resumed at 30 C in the presence of these drugs. (Fig. 3). This is not due to a direct inhibition of DNA synthesis by rifampin or streptolydigin, for both these drugs and chloramphenicol permit an increase in DNA of about 40% when added to an exponentially growing culture (Fig. 3). After a previous period at 45 C, the initiation of new replication round, which can occur at 30 C in the presence of chloramphenicol, is prevented by the inhibitors of RNA synthesis. This demonstrates that the initiation of DNA replication in *B. subtilis* requires a transcriptional step, even when all of the proteins needed for the initiation process are already present.

DISCUSSION

Reversibility of the inhibition of DNA initiation. When mutant Ts37 is returned to 30 C, after a period at 45 C, DNA synthesis is resumed, even in the presence of chloramphenicol. A doubling of the initial amount of DNA is thus permitted, suggesting replication of the whole chromosome. This property, absent in several mutants altered in the process of initiation of replication (1, 9, 11) was also found in

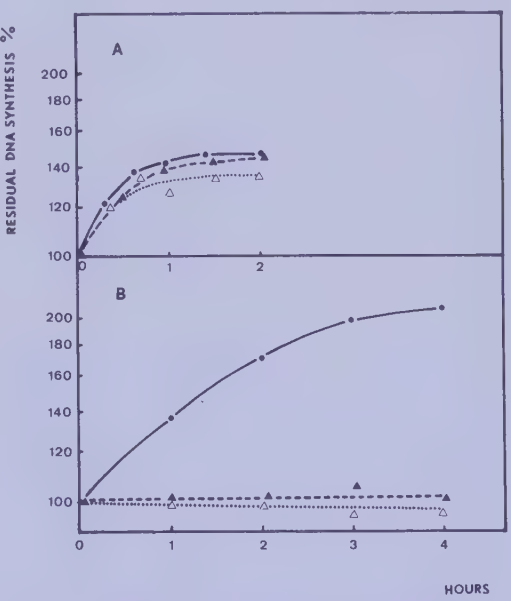

FIG. 3. *Effect of rifampin and streptolydigin on DNA synthesis. An exponentially growing culture of the mutant Ts37 incubated at 30 C in the presence of ^{14}C-thymine (0.08 μCi per 5 μg per ml) for 10 generations was divided into several parts. A, Three parts received either chloramphenicol (150 μg/ml), rifampin (500 μg/ml), or streptolydigin (250 μg/ml), and were further incubated at 30 C. B, Three other parts of the culture were shifted to 45 C; after 60 min, they received either chloramphenicol (150 μg/ml), rifampin (500 μg/ml), or streptolydigin (250 μg/ml) and, 5 min later, they were returned to 30 C. The incorporation of ^{14}C-thymine into trichloroacetic acid-precipitable material was followed as described in Materials and Methods. Values are normalized to the value observed at the time of addition of the inhibitor (curves A) or to the value at the time of the shift back to 30 C, taken as 100 (curves B). Chloramphenicol (●); rifampin (▲); streptolydigin (△).*

some mutants of *E. coli* (2, 23, 30). The formation of exclusively hybrid DNA, shown in Fig. 2, and the Ade/Met ratio observed for this DNA permit us to conclude that a new round is initiated and completely replicated under these conditions. Moreover, a large extent of DNA repair may be excluded for there is no important displacement of the hybrid peak towards the light density; this is in agreement with the stability of DNA observed at 45 C (unpublished data). Our results suggest that a protein required for initiation is synthesized at 45 C in an inactive form and can be activated at 30 C. For all of the bacteria to acquire this protein and the capacity to initiate a new round of replication, a minimal period at 45 C is required, which is about a generation time.

Reinitiation of DNA replication is limited to one cycle. Even after a prolonged exposure at 45 C, DNA synthesis never exceeds a doubling, when the temperature is lowered in the presence of chloramphenicol, thus suggesting that only a limited amount of initiation material is available after a period at 45 C. This particular point will be further studied in another paper (S. Laurent, manuscript in preparation). Such a limit might reflect an aspect of the regulation of initiation frequency. Based on previous concepts (10, 17), positive (7, 8) or negative (20, 22) regulation mechanisms have been proposed. They cannot presently be distinguished by our data.

Requirement for a transcriptional step. The initiation process is sensitive to rifampin and streptolydigin, but resistant to chloramphenicol (Fig. 3). These results suggest that initiation of DNA replication in *B. subtilis* requires a transcriptional step, in addition to one or more specific initiation proteins. Such a requirement has been shown for DNA replication in other organisms and has been variously interpreted. In the phages M13 and ΦX174, a short segment of ribonucleic acid, covalently linked to DNA, is considered to be a primer for DNA replication (4, 28, 29). In *E. coli*, a structural role of this RNA has been proposed (12). The replication proteins would be assembled around an RNA core, thus forming the replication complex and identifying the chromosome origin. In connection with this hypothesis, it is interesting to note the evidence, given in *E. coli*, of an RNA core responsible for the folded structure of the chromosome (25, 31).

ACKNOWLEDGMENTS

I am much indebted to J. Legault-Demare for helpful discussions and to F. Espardellier, G. Lindahl, and P. Schaeffer for reviewing the manuscript. I would like to thank F. Vannier for excellent assistance during this work.

LITERATURE CITED

1. Abe, M., and J. I. Tomizawa. 1971. Chromosome replication in *Escherichia coli* K12 mutant affected in the process of DNA initiation. Genetics **69**:1–15.
2. Beyersmann, D., M. Schlicht, and H. Schuster. 1971. Temperature sensitive initiation of DNA replication in a mutant of *Escherichia coli* K12. Mol. Gen. Genet. **111**:145–158.
3. Bollum, F. J. 1966. Filter paper disc techniques for assaying radioactive macromolecules, p. 296–300. *In* G. L. Cantoni and D. R. Davies (ed.), Procedures in nucleic acid research. Harper and Row Publishers, New York.
4. Brutlag, D., R. Schekman, and A. Kornberg. 1971. A possible role for RNA polymerase in the initiation of M13 DNA synthesis. Proc. Nat. Acad. Sci. U.S.A., **68**:2826–2829.
5. Cassani, G., R. R. Burgess, and H. M. Goodman. 1970. Streptolydigin inhibition of RNA polymerase. Cold Spring Harbor Symp. Quant. Biol. **35**:59–63.
6. Cooper, S., and C. E. Helmstetter. 1968. Chromosome replication and the cell division cycle of *Escherichia coli* B/r. J. Mol. Biol. **31**:519–540.
7. Donachie, W. D. 1968. Relationship between cell size and time of initiation of DNA replication. Nature (London) **219**:1077–1079.
8. Helmstetter, C. E., S. Cooper, O. Pierucci, and E. Revelas. 1968. On the bacterial life sequence. Cold Spring Harbor Symp. Quant. Biol. **33**:809–822.
9. Hirota, Y., J. Mordoh, and F. Jacob. 1970. On the process of cellular division in *Escherichia coli*. III. Thermosensitive mutants of *Escherichia coli* altered in the process of DNA initiation. J. Mol. Biol. **53**:369–387.
10. Jacob, F., S. Brenner, and F. Cuzin. 1963. On the regulation of DNA replication in bacteria. Cold Spring Harbor Symp. Quant. Biol. **28**:329–348.
11. Kuempel, P. L. 1969. Temperature-sensitive initiation of chromosome replication in a mutant of *Escherichia coli*. J. Bacteriol. **100**:1302–1310.
12. Lark, K. G. 1972. Evidence for the direct involvement of RNA in the initiation of DNA replication in *Escherichia coli* 15 T^-. J. Mol. Biol. **64**:47–60.
13. Lark, K. G., and H. Renger. 1969. Initiation of DNA replication in *Escherichia coli* 15 T^-: chronological dissection of three physiological processes required for initiation. J. Mol. Biol. **42**:221–235.
14. Lark, K. G., T. Repko, and E. J. Hoffman. 1963. The effect of amino acid deprivation on subsequent deoxyribonucleic acid replication. Biochim. Biophys. Acta **76**:9–24.
15. Laurent, S. J., and F. S. Vannier. 1973. Temperature-sensitive initiation of chromosome replication in a mutant of *Bacillus subtilis*. J. Bacteriol. **114**:474–484.
16. Maaløe, O., and P. C. Hanawalt. 1961. Thymine deficiency and the normal DNA replication cycle. J. Mol. Biol. **3**:144–155.
17. Maaløe, O., and N. O. Kjeldgaard. 1966. The regulation of DNA synthesis, p. 154–187. *In* Control of macromolecular synthesis. W. A. Benjamin Inc., New York.
18. Marmur, J. 1961. A procedure for the isolation of deoxyribonucleic acid from micro-organisms. J. Mol. Biol. **3**:208–218.
19. Oishi, M., H. Yoshikawa, and N. Sueoka. 1964. Synchronous and dichotomous replications of the *Bacillus subtilis* chromosome during spore germination. Nature (London) **204**:1069–1073.
20. Pritchard, R. H., P. T. Barth, and J. Collins. 1969. Control of DNA synthesis in bacteria. Symp. Soc. Gen. Microbiol. **19**:263–297.
21. Pritchard, R. H., and K. G. Lark. 1964. Induction of replication by thymine starvation at the chromosome origin in *Escherichia coli*. J. Mol. Biol. **9**:288–307.

22. Rosenberg, B. H., L. J. Cavalieri, and G. Ungers. 1969. The negative control mechanism for *E. coli* DNA replication. Proc. Nat. Acad. Sci. U.S.A. **63:** 1410–1417.
23. Schubach, W. H., J. D. Whitmer, and C. I. Davern. 1973. Genetic control of DNA initiation in *Escherichia coli*. J. Mol. Biol. **74:**205–221.
24. Spizizen, J. 1958. Transformation of biochemically deficient strains of *Bacillus subtilis* by deoxyribonucleate. Proc. Nat. Acad. Sci. U.S.A. **44:**1072–1078.
25. Stonington, O. G., and D. E. Pettijohn. 1971. The folded genome of *Escherichia coli* isolated in a protein-DNA-RNA complex. Proc. Nat. Acad. Sci. U.S.A. **68:**6–9.
26. Yoshikawa, H., and H. Haas. 1968. On the regulation of the initiation of DNA replication in bacteria. Cold Spring Harbor Symp. Quant. Biol. **33:**843–855.
27. Wehrli, W., and M. Staehelin. 1971. Actions of the rifamycins. Bacteriol. Rev. **35:**290–309.
28. Westergaard, O., D. Brutlag, and A. Kornberg. 1973. Initiation of deoxyribonucleic acid synthesis. IV. Incorporation of the ribonucleic acid primer into the phage replicative form. J. Biol. Chem. **248:**1361–1364.
29. Wickner, W., D. Brutlag, R. Schekman, and A. Kornberg. 1972. RNA synthesis initiates *in vitro* conversion of M13 DNA to its replicative form. Proc. Nat. Acad. Sci. U.S.A. **69:**965–969.
30. Wolf, B. 1972. The characteristics and genetic map location of a temperature-sensitive DNA mutant of *E. coli* K12. Genetics **72:**569–593.
31. Worcel, A., and E. Burgi. 1972. On the structure of the folded chromosome of *Escherichia coli*. J. Mol. Biol. **71:**127–147.